REMARKS

Status of the claims

Upon entry of these remarks, claims 1-359 will be pending in this application. Claims 26-359 have been provisionally elected, *with traverse*.

New claims 26-359, which correspond to provisionally elected Group II (cancelled claims 17, 18, 20, and 22) have been added to more particularly and distinctly point out and distinctly claim the subject matter Applicants regard as the invention. Support for the newly added claims is found throughout the specification as filed, and no new matter had been introduced.

More particularly, support for new claims directed to polypeptides, polypeptide fragments and % identity to polypeptides can be found, for example, on pages 11-12, 93, and 179. Support for new claims directed to N- and C-terminal deletions, % identity to N- and C- terminal deletions can be found, for example, on pages 101-116. Support for new claims directed to heterologous polypeptides including Fc domains can be found, for example, on pages 56 and 75. Support for new claims directed to methods of producing polypeptides can be found, for example, on pages 69-80. Support for new claims directed to compositions can be found, for example, on pages 282-298. Support for new claims directed to polypeptides that are modulating cell proliferation and differentiation can be found, for example, on pages 65-66. And Support for new claims directed to polypeptides that are cytotoxic to Neutrokine-alpha receptor bearing cells can be found, for example, on pages 253-254. Thus, no new matter has been added by way of amendment.

Provisional Election with Traverse

The Examiner has required restriction of the claims into three groups - Group I drawn to a nucleic acid molecule encoding Neutokine-alpha protein, a vector, a host cell and a method of making the protein represented by claims 1-16; Group II drawn to a Neutrokine-alpha polypeptide represented by claim 17, 18, 20, and 22; and Group III drawn to an antibody to Neutrokine-alpha.

In accordance with 37 C.F.R. § 1.143, Applicants hereby provisionally elect Group II (claims 17, 18, 20, and 22, new claims 26-359), *with traverse*. Applicants reserve the

right to file one or more divisional applications directed to the non-elected inventions should the restriction requirement be made final.

Applicants respectfully traverse the restriction requirement as it applies to Groups I, II, and III. Even assuming, for the sake of argument, that more than a single patentably distinct invention appears in an application, restriction remains improper unless it can be shown that the search and examination of each group would entail a "serious burden" (*see* M.P.E.P. § 803). In the present situation, no such showing has been made. Indeed, no arguments have been made explaining why it would impose an undue burden to examine the polynucleotide, polypeptide, and antibody claims together.

Applicants submit that a search of the polynucleotide claims would clearly provide useful information for the polypeptide claims. For example, in many if not most publications, where a published nucleotide sequence contains an open reading frame, the authors also include, as a matter of routine, the deduced amino acid sequence. Thus, the searches for polynucleotides and polypeptides commonly overlap. Even in the relatively uncommon case where a publication contains a nucleotide sequence which is not accompanied by the corresponding deduced amino acid sequence, it is routine for one to determine the corresponding amino acid sequence. Moreover, a search for Neutrokine-α polypeptides would include, also as a matter of routine, a search for antibodies specific for Neutrokine-α. Thus, the search and examination of a polynucleotide, corresponding deduced polypeptide sequences, and antibodies specific for the corresponding deduced polypeptide sequences would not entail a serious burden.

Thus, in view of M.P.E.P. § 803, all of the claims should be searched and examined in the subject application. Applicants respectfully request that the above-made remarks be entered and made of record in the file history of the instant application.

Amendments to the Drawings

In Formal Drawing 7A-1, the following minor changes have been made compared to the originally filed informal version of Figure 7: 1) the line demarcating the transmembrane region has been shortened such that it extends only over the L at position 72 and no further; 2) the amino acid number designation "77", at the end of the first line of LT-alpha sequence has been changed to "76"; and 3) the "G" at position 197 of

Neutrokine-alpha sequence was changed from being white on black to being black on white. Copies of the original drawing are attached with these changes marked in red.

The amendment of the length of the line demarcating the transmembrane region brings the drawing into conformity with the description of the transmembrane region as it is defined in the specification in several locations. See for example page 11, line 32 to page 12, line 2, where the transmembrane region is defined as amino acids 47 to 72 of the Neutrokine-alpha polypeptide. Thus, this amendment introduces no new matter and is fully supported by the specification.

The amendment of the amino acid number designation corrects the numbering of the sequence such that it agrees with the numbering of the sequence of Lymphotoxin-alpha given in Gray, *Nature* 312,721-724 (1984). The Gray *et al.* Nature article is cited on page 336, lines 20-21 of the specification in reference to Figure 7A. GenBank Reports for Accession Numbers CAA25649 and P01374 are submitted in support of this amendment. CAA25649 is the original GenBank submission for the Gray *et al.* article. Because the Gray GenBank report does not identify the lymphotoxin (LT) as LT-alpha, GenBank report P01374, which also cites the Gray *et al.* reference, is also included. Thus, this amendment introduces no new matter and is fully supported by the specification.

The shading of certain amino acid residues in Figure 7 indicates which amino acid residues match the consensus sequence. The amendment of the "G" at position 197 of Neutrokine-alpha sequence corrects the shading because a serine (S), and not a glycine (G) residue is the consensus residue at this position. Support for this amendment is found in Figure 2B (Formal Drawing Figure 2C) where the G at position 197 of the Neutrokine alpha polypeptide is not shaded. Thus, this amendment introduces no new matter and is fully supported by the specification.

CONCLUSION

Applicants respectfully request that the remarks above be entered and made of record in the file history of the instant application.

Respectfully submitted,

Dated: August 14, 2001

Kenley K. Hoover (Reg. No. 40,302)
Attorney for Applicants

Human Genome Sciences, Inc.
9410 Key West Avenue
Rockville, MD 20850
Telephone: (301) 610-5771

KKH/MS/cmp

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Application of: Yu et al.

Application No.: 09/507,968 Art Unit: 1646

Filed: February 22, 2000 Examiner: M. Ceperley

For: Neutrokine-alpha and Neutrokine-alpha Splice Variant Atty Docket No.: PF343P3

VERSION WITH MARKINGS TO SHOW CHANGES MADE

Amendments are indicated in boldfaced text with insertions indicated by underlining and deletions indicated by strikeout text.

Please replace the section entitled "Brief Description of the Figures" beginning on line 7 of page 20 and ending on page 25, line 2, with the following amended section:

Brief Description of the Figures

The following drawings are illustrative of embodiments of the invention and are not meant to limit the scope of the invention as encompassed by the claims.

Figures 1A and 1B ~~shows~~ show the nucleotide (SEQ ID NO:1) and deduced amino acid (SEQ ID NO:2) sequences of Neutrokine-alpha. Amino acids 1 to 46 represent the predicted intracellular domain, amino acids 47 to 72 the predicted transmembrane domain (the double-underlined sequence), and amino acids 73 to 285, the predicted extracellular domain (the remaining sequence). Potential asparagine-linked glycosylation sites are marked in Figures 1A and 1B with a bolded asparagine symbol (N) in the Neutrokine-alpha amino acid sequence and a bolded pound sign (#) above the first nucleotide encoding that asparagine residue in the Neutrokine-alpha nucleotide sequence. Potential N-linked glycosylation sequences are found at the following locations in the

Neutrokine-alpha amino acid sequence: N-124 through Q-127 (N-124, S-125, S-126, Q-127) and N-242 through C-245 (N-242, N-243, S-244, C-245).

Regions of high identity between Neutrokine-alpha, Neutrokine-alphaSV, TNF-alpha, TNF-beta, LT-beta, and the closely related Fas Ligand (an alignment of these sequences is presented in ~~Figure 2~~ **Figures 2A, 2B, 2C and 2D**) are underlined in Figures 1A and 1B. These regions are not limiting and are labeled as Conserved Domain (CD)-I, CD-II, CD-III, CD-IV, CD-V, CD-VI, CD-VII, CD-VIII, CD-IX, CD-X, and CD-XI in Figures 1A and 1B.

Figures ~~2A and 2B~~ **2A, 2B, 2C and 2D** show the regions of identity between the amino acid sequences of Neutrokine-alpha (SEQ ID NO:2) and Neutrokine-alphaSV (SEQ ID NO:19), and TNF-alpha ("TNFalpha" in Figures ~~2A and 2B~~ **2A, 2B, 2C and 2D**; GenBank No. Z15026; SEQ ID NO:3), TNF-beta ("TNFbeta" in Figures ~~2A and 2B~~ **2A, 2B, 2C and 2D**; GenBank No. Z15026; SEQ ID NO:4), Lymphotoxin-beta ("LTbeta" in Figures ~~2A and 2B~~ **2A, 2B, 2C and 2D**; GenBank No. L11016; SEQ ID NO:5), and FAS ligand ("FASL" in Figures 2A**, 2B, 2C and 2D**; GenBank No. U11821; SEQ ID NO:6), determined by the "MegAlign" routine which is part of the computer program called "DNA*STAR." Residues that match the consensus are shaded.

Figure 3 shows an analysis of the Neutrokine-alpha amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown, as predicted for the amino acid sequence of SEQ ID NO:2 using the default parameters of the recited computer programs. In the "Antigenic Index - Jameson-Wolf" graph, the indicate location of the highly antigenic regions of Neutrokine-alpha i.e., regions from which epitope-bearing peptides of the invention may be obtained. Antigenic polypeptides include from about Phe-115 to about Leu-147, from about Ile-150 to about Tyr-163, from about Ser-171 to about Phe-194, from about Glu-223 to about Tyr-246, and from about Ser-271 to about Phe-278, of the amino acid sequence of SEQ ID NO:2.

The data presented in Figure 3 are also represented in tabular form in Table I. The columns are labeled with the headings "Res", "Position", and Roman Numerals I-XIV. The column headings refer to the following features of the amino acid sequence presented in Figure 3, and Table I: "Res": amino acid residue of SEQ ID NO:2 and Figures 1A and 1B; "Position": position of the corresponding residue within SEQ ID NO:2 and Figures 1A and 1B; I: Alpha, Regions - Garnier-Robson; II: Alpha, Regions - Chou-Fasman; III: Beta,

Regions - Garnier-Robson; IV: Beta, Regions - Chou-Fasman; V: Turn, Regions - Garnier-Robson; VI: Turn, Regions - Chou-Fasman; VII: Coil, Regions - Garnier-Robson; VIII: Hydrophilicity Plot - Kyte-Doolittle; IX: Hydrophobicity Plot - Hopp-Woods; X: Alpha, Amphipathic Regions - Eisenberg; XI: Beta, Amphipathic Regions - Eisenberg; XII: Flexible Regions - Karplus-Schulz; XIII: Antigenic Index - Jameson-Wolf; and XIV: Surface Probability Plot - Emini.

Figures 4A, 4B, and 4C show the alignment of the Neutrokine-alpha nucleotide sequence determined from the human cDNA deposited in ATCC No. 97768 with related human cDNA clones of the invention which have been designated HSOAD55 (SEQ ID NO:7), HSLAH84 (SEQ ID NO:8) and HLTBM08 (SEQ ID NO:9).

Figures 5A and 5B ~~shows~~ show the nucleotide (SEQ ID NO:18) and deduced amino acid (SEQ ID NO:19) sequences of the Neutrokine-alphaSV protein. Amino acids 1 to 46 represent the predicted intracellular domain, amino acids 47 to 72 the predicted transmembrane domain (the double-underlined sequence), and amino acids 73 to 266, the predicted extracellular domain (the remaining sequence). Potential asparagine-linked glycosylation sites are marked in Figures 5A and 5B with a bolded asparagine symbol (N) in the Neutrokine-alphaSV amino acid sequence and a bolded pound sign (#) above the first nucleotide encoding that asparagine residue in the Neutrokine-alphaSV nucleotide sequence. Potential N-linked glycosylation sequences are found at the following locations in the Neutrokine-alphaSV amino acid sequence: N-124 through Q-127 (N-124, S-125, S-126, Q-127) and N-223 through C-226 (N-223, N-224, S-225, C-226). Antigenic polypeptides include from about Pro-32 to about Leu-47, from about Glu-116 to about Ser-143, from about Phe-153 to about Tyr-173, from about Pro-218 to about Tyr-227, from about Ala-232 to about Gln-241; from about Ile-244 to about Ala-249; and from about Ser-252 to about Val-257 of the amino acid sequence of SEQ ID NO:19.

Regions of high identity between Neutrokine-alpha, Neutrokine-alphaSV, TNF-alpha, TNF-beta, LT-beta, and the closely related Fas Ligand (an aligment of these sequences is presented in ~~**Figure 2**~~ **Figures 2A, 2B, 2C and 2D**) are underlined in Figures 1A and 1B. These conserved regions (of Neutrokine-alpha and Neutrokine-alphaSV) are labeled as Conserved Domain (CD)-I, CD-II, CD-III, CD-V, CD-VI, CD-VII, CD-VIII, CD-IX, CD-X, and CD-XI in Figures 5A and 5B. Neutrokine-alphaSV does not contain the sequence of CD-IV described in the legend of Figures 1A and 1B.

An additional alignment of the Neutrokine-alpha polypeptide sequence (SEQ ID NO:2) with APRIL, TNF alpha, and LT alpha is presented in ~~Figure 7A~~ **Figures 7A-1 and 7A-2**. ~~Figure 7A~~ **Figures 7A-1 and 7A-2**, beta sheet regions are indicated as described below in the ~~Figure 7A~~ legend **to Figures 7A-1 and 7A-2**.

Figure 6 shows an analysis of the Neutrokine-alphaSV amino acid sequence. Alpha, beta, turn and coil regions; hydrophilicity and hydrophobicity; amphipathic regions; flexible regions; antigenic index and surface probability are shown, as predicted for the amino acid sequence of SEQ ID NO:19 using the default parameters of the recited computer programs. The location of the highly antigenic regions of the Neutrokine-alpha protein, i.e., regions from which epitope-bearing peptides of the invention may be obtained is indicated in the "Antigenic Index - Jameson-Wolf" graph. Antigenic polypeptides include, but are not limited to, a polypeptide comprising amino acid residues from about Pro-32 to about Leu-47, from about Glu-116 to about Ser-143, from about Phe-153 to about Tyr-173, from about Pro-218 to about Tyr-227, from about Ser-252 to about Thr-258, from about Ala-232 to about Gln-241; from about Ile-244 to about Ala-249; and from about Ser-252 to about Val-257, of the amino acid sequence of SEQ ID NO:19.

The data shown in Figure 6 can be easily represented in tabular format similar to the data shown in Table I. Such a tablular representation of the exact data disclosed in Figure 6 can be generated using the MegAlign component of the DNA*STAR computer sequence analysis package set on default parameters. This is the identical program that was used to generate Figures 3 and 6 of the present application.

Figure 7A. The amino-acid sequence of Neutrokine-alpha and alignment of its predicted ligand-binding domain with those of APRIL, TNF-alpha, and LT-alpha (specifically, amino acid residues 115-250 of the human APRIL polypeptide (SEQ ID NO:20; ATCC Accession No. AF046888), amino acid residues 88-233 of TNF alpha (SEQ ID NO:3; GenBank Accession No. Z15026), and LT alpha ((also designated TNF-beta) amino acid residues 62-205 of SEQ ID NO:4; GenBank Accession No. Z15026)). The predicted membrane-spanning region of Neutrokine-alpha is indicated and the site of cleavage of Neutrokine-alpha is depicted with an arrow. Sequences overlaid with lines (A thru H) represent predicted beta-pleated sheet regions.

Figure 7B. Expression of Neutrokine-alpha mRNA. Northern hybridization analysis was performed using the Neutrokine-alpha orf as a probe on blots of poly (A)+

RNA (Clonetech) from a spectrum of human tissue types and a selection of cancer cell lines. A 2.6 kb Neutrokine-alpha mRNA was detected at high levels in placenta, heart, lung, fetal liver, thymus, and pancreas. The 2.6 kb Neutrokine-alpha mRNA was also detected in HL-60 and K562 cell lines.

Figures ~~8A and 8B~~ 8A-C. Neutrokine-alpha expression increases following activation of human monocytes by IFN-gamma. **~~Figure 8A~~ Figures 8A and 8B.** Flow cytometric analysis of Neutrokine-~~alpa~~ **alpha** protein expression on *in vitro* cultured monocytes. Purified monocytes were cultured for 3 days in presence or absence of IFN-gamma (100 U/ml). Cells were then stained with a Neutrokine-alpha-specific mAb (2E5) (solid lines) or an isotype-matched control (IgG1) (dashed lines). Comparable results were obtained with monocytes purified from three different donors in three independent experiments. **Figure ~~8B~~ 8C.** Neutrokine-alpha-specific TaqMan primers were prepared and used to assess the relative Neutrokine-alpha mRNA expression levels in unstimulated and IFN-gamma (100 U/mL) treated monocytes. Nucleotide sequences of the TaqMan primers are as follows: (a) Probe: 5'-CCA CCA GCT CCA GGA GAA GGC AAC TC-3' (SEQ ID NO:24); (b) 5' amplification primer: 5'-ACC GCG GGA CTG AAA ATC T-3' (SEQ ID NO:25); and (c) 3' amplification primer: 5'-CAC GCT TAT TTC TGC TGT TCT GA-3' (SEQ ID NO:26).

Figures 9A and 9B. Neutrokine-alpha is a potent B lymphocyte stimulator. **Figure 9A.** The biological activity of Neutrokine-alpha was assessed in a standard B-lymphocyte co-stimulation assay utilizing *Staphylococcus aureus cowan 1* SAC as the priming agent. SAC alone yielded background counts of 1427 +/- 316. Values are reported as mean +/- standard deviation of triplicate wells. Similar results were obtained using recombinant Neutrokine-alpha purified from stable CHO transfectants and transiently transfected HEK 293T cells. **Figure 9B.** Proliferation of tonsillar B cells with Neutrokine-alpha and co-stimulation with anti-IgM. The bioassay was performed as described for SAC with the exception that individual wells were pre-coated with goat anti-human IgM antibody at 10 micrograms/mL in PBS.

Figures ~~10A and 10B~~ 10A, 10B, 10C, 10D, 10E, 10F, and 10G. Neutrokine-alpha receptor expression among normal human peripheral blood mononuclear cells and tumor cell lines. **~~Figure 10A~~ Figures 10A, 10B, 10C, 10D and 10E.** Human peripheral blood nucleated cells were obtained from normal volunteers and isolated by density gradient centrifugation. Cells were stained with biotinylated Neutrokine-alpha followed

by PE-conjugated streptavidin and FITC or PerCP coupled mAbs specific for CD3, CD20, CD14, CD56, and CD66b. Cells were analyzed on a Becton Dickinson FACScan using the CellQuest software. Data represent one of four independent experiments. ~~**Figure 10B**~~ **Figures 10F and 10G.** Neutrokine-alpha binding to histiocytic cell line U-937 and the myeloma line IM-9.

Figures ~~11A, 11B and 11C~~ 11A, 11B, 11C, 11D, 11E and 11F. *In vivo* effects of Neutrokine-alpha administration in BALB/cAnNCR mice. **Figure 11A.** Formalin-fixed spleens were paraffin embedded and 5 micrometer sections stained with hematoxylin and eosin (upper panels). The lower panels are sections taken from the same animals stained with anti-CD45R(B220) mAb and developed with horseradish-peroxidase coupled rabbit anti-rat Ig (mouse adsorbed) and the substrate diaminobenzidine tetrahydrochloride (DAB). Slides were counter-stained with Mayer's hematoxylin. CD45R(B220) expressing cells appear brown. ~~**Figure 11B**~~ **Figures 11B and 11C.** Flow cytometric analyses of normal (left panel) and Neutrokine-alpha-treated (right panel) stained with PE-CD45R(B220) and FITC-ThB (Ly6D). ~~**Figure 11C**~~ **Figures 11D, 11E and 11F.** Serum IgM, IgG, and IgA levels in normal and Neutrokine-alpha treated mice.

Please replace the paragraph spanning lines 21 to27 of page 25, with the following amended paragraph:

The Neutrokine-alpha and Neutrokine-alpha polypeptides of the present invention share sequence homology with the translation products of the human mRNAs for TNF-alpha, TNF-beta, LTbeta, Fas ligand, APRIL, and LTalpha. (See, Figures ~~2A, 2B, and 7A~~ 2A, 2B, 2C, 2D, 7A-1 and 7A-2). As noted above, TNF-alpha is thought to be an important cytokine that plays a role in cytotoxicity, necrosis, apoptosis, costimulation, proliferation, lymph node formation, immunoglobulin class switch, differentiation, antiviral activity, and regulation of adhesion molecules and other cytokines and growth factors.

Please replace the paragraph spanning lines 3 to 16 of page 42, with the following amended paragraph:

Accordingly, specific embodiments of the invention are directed to polynucleotides encoding polypeptides which comprise, or alternatively consist of, the amino acid sequence of beta pleated sheet region A, A', B, B', C, D, E, F, G, or H disclosed in ~~Figure 7A~~ **Figures 7A-1 and 7A-2** and described in Example 6. Additional embodiments of the invention are directed to polynucleotides encoding Neutrokine-alpha polypeptides which comprise, or alternatively consist of, any combination of 1, 2, 3, 4, 5, 6, 7, 8, 9 or all 10 of beta pleated sheet regions A-H disclosed in ~~Figure 7A~~ **Figures 7A-1 and 7A-2** and described in Example 6. Additional preferred embodiments of the invention are directed to polypeptides which comprise, or alternatively consist of, the Neutrokine-alpha amino acid sequence of beta pleated sheet region A, A', B, B', C, D, E, F, G, or H disclosed in ~~Figure 7A~~ **Figures 7A-1 and 7A-2** and described in Example 6. Additional embodiments of the invention are directed Neutrokine-alpha polypeptides which comprise, or alternatively consist of, any combination of 1, 2, 3, 4, 5, 6, 7, 8, 9 or all 10 of beta pleated sheet regions A through H disclosed in ~~Figure 7A~~ **Figures 7A-1 and 7A-2** and described in Example 6.

Please replace the paragraph spanning lines 17 to 33 of page 42, with the following amended paragraph:

In certain other preferred embodiments, polynucleotides of the invention comprise, or alternatively consist of, nucleotide residues 34-57, 118-123, 133-141, 151-159, 175-216, 232-255, 280-315, 328-357, 370-393, and/or 430-456 of SEQ ID NO:21. Polypeptides encoded by these polynucleotides are also encompassed by the invention. These polynucleotide and polypeptide fragments correspond to the predicted beta-pleated sheet regions shown in ~~Figure 7A~~ **Figures 7A-1 and 7A-2**. In certain embodiments, polynucleotides of the invention comprise, or alternatively consist of, a polynucleotide sequence at least 90%, 95%, 96%, 97%, 98% or 99% identical to the polynucleotide sequence encoding one, two, three, four, five, six, seven, eight, nine or ten of the beta-pleated sheet regions described above. The present invention also encompasses the above polynucleotide sequences fused to a heterologous polynucleotide sequence. Polypeptides encoded by these polynucleotide sequences are also encompassed by the invention. In

another embodiment, the invention provides an isolated nucleic acid molecule comprising a polynucleotide which hybridizes under stringent hybridization conditions to one, two, three, four, five, six, seven, eight, nine or ten of the beta-pleated sheet polynucleotides of the invention described above. The meaning of the phrase "stringent conditions" as used herein is described *infra*.

Please replace the paragraph spanning lines 1 to 6 of page 43, with the following amended paragraph:

In further preferred embodiments, polynucleotides of the invention comprise, or alternatively consist of, nucleotide residues 576-599, 660-665, 675-683, 693-701, 717-758, 774-803, 822-857, 870-899, 912-935, and/or 972-998 of SEQ ID NO:1. Polypeptides encoded by these polynucleotide fragments are also encompassed by the invention. These polynucleotide and polypeptide fragments correspond to the predicted beta-pleated sheet regions shown in ~~**Figure 7A**~~ **Figures 7A-1 and 7A-2**.

Please replace the paragraph spanning lines 7 to 12 of page 43, with the following amended paragraph:

In additional preferred embodiments, polynucleotides of the invention comprise, or alternatively consist of, nucleotide residues 457-462, 472-480, 490-498, 514-555, 571-600, 619-654, 667-696, 699-732, and/or 769-795 of SEQ ID NO:18. Polypeptides encoded by these polynucleotide fragments are also encompassed by the invention. These polynucleotide and polypeptide fragments correspond to the predicted beta-pleated sheet regions shown in ~~**Figure 7A**~~ **Figures 7A-1 and 7A-2**.

Please replace the paragraph spanning lines 13 to 21 of page 43, with the following amended paragraph:

In yet further preferred embodiments, polynucleotides of the invention comprise, or alternatively consist of, nucleotide residues 124-129, 139-147, 157-165, 181-222, 238-267, 286-321, 334-363, 376-399, and/or 436-462 of SEQ ID NO:22. Polypeptides encoded by these polynucleotide fragments are also encompassed by the invention. These polynucleotide and polypeptide fragments correspond to the predicted beta-pleated sheet regions shown in ~~Figure 7A~~ **Figures 7A-1 and 7A-2**. Polypeptides comprising, or alternatively, consisting of the amino acid sequence of any combination of one, two, three, four, five, six, seven, eight, nine, ten, or all of these regions are encompassed by the invention.

Please replace the paragraph spanning lines 22 to 24 of page 46, with the following amended paragraph:

It is believed one or more of the beta pleated sheet regions of Neutrokine-alpha disclosed in ~~Figure 7A~~ **Figures 7A-1 and 7A-2** is important for dimerization and also for interactions between Neutrokine-alpha and its ligands.

Please replace the paragraph spanning lines 27 to 32 of page 66, with the following amended paragraph:

In certain preferred embodiments, "a polypeptide having Neutrokine-alpha polypeptide functional activity" (e.g., biological activity) and "a polypeptide having Neutrokine-alphaSV polypeptide functional activity" (e.g., biological activity) includes polypeptides that also exhibit any of the same B cell (or other cell type) modulatory (particularly immunomodulatory) activities described in Figures ~~8A, 8B, 9A, 9B, 10, 11, 12A, and 12B~~ **8A, 8B, 9A, 9B, 10A, 10B, 10C, 10D, 10E, 10F, 10G, 11A, 11B, and 11C**, and in Example 6.

Please replace the paragraph spanning lines 8 to 29 of page 88, with the following amended paragraph:

Certain additional embodiments of the invention encompass polypeptide fragments comprising, or alternatively consisting of, the predicted beta-pleated sheet regions identified in ~~Figure 7A~~ **Figures 7A-1 and 7A-2**. These polypeptide fragments of the invention comprise, or alternatively consist of, amino acid residues Gln-144 to Ala-151, Phe-172 to Lys-173, Ala-177 to Glu-179, Asn-183 to Ile-185, Gly-191 to Lys-204, His-210 to Val-219, Leu-226 to Pro-237, Asn-242 to Ala-251, Gly-256 to Ile-263 and/or Val-276 to Leu-284 of SEQ ID NO:2. In another, nonexclusive embodiment, these polypeptide fragments of the invention also comprise, or alternatively consist of, amino acid residues Phe-153 to Lys-154, Ala-158 to Glu-160, Asn-164 to Ile-166, Gly-172 to Lys-185, His-191 to Val-200, Leu-207 to Pro-218, Asn-223 to Ala-232, Gly-237 to Ile-244 and/or Val-257 to Leu-265 of SEQ ID NO:19; and amino acid residues Phe-42 to Lys-43, Ala-47 to Glu-49, Asn-53 to Ile-55, Gly-61 to Pro-74, His-80 to Val-89, Leu-96 to Pro-107, Asn-112 to Ala-121, Gly-126 to Ile-133 and/or Asp-146 to Leu-154 of SEQ ID NO:23. In further nonexclusive embodiments, these polypeptide fragments of the invention also comprise, or alternatively consist of, amino acid residues Gln-78 to Ala-85; Phe-106 to Lys-107, Ala-111 to Glu-113, Asn-117 to Ile-119, Gly-125 to Lys-138, His-144 to Val-153, Leu-160 to Pro-171, Asn-176 to Ala-185, Gly-190 to Ile-197 and/or Val-210 to Leu-218 of SEQ ID NO:28; and amino acid residues Gln-78 to Ala-85; Phe-106 to Lys-107, Ala-111 to Glu-113, Asn-117 to Ile-119, Gly-125 to Lys-138, His-144 to Val-153, Leu-160 to Pro-171, Asn-176 to Ala-185, Gly-190 to Ile-197 and/or Val-210 to Leu-218 of SEQ ID NO:30. Polynucleotides encoding these polypeptide fragments are also provided.

Please replace the paragraph spanning lines 1 to 16 of page 175, with the following amended paragraph:

Since Neutrokine-alpha is a member of the TNF-related protein family, to modulate rather than completely eliminate functional activities (e.g., biological activities) of Neutrokine-alpha, mutations may be made in sequences encoding amino acids in the TNF conserved domain, i.e., in positions Gly-191 through Leu-284 of Figures 1A and 1B (SEQ ID NO:2), more preferably in residues within this region which are not conserved in

all, most or several members of the TNF family (e.g., TNF-alpha, TNF-beta, LT-beta, and Fas Ligand) (see e.g., Figures ~~2A-B~~ **2A, 2B, 2C and 2D**). By making a specific mutation in Neutrokine-alpha in the position where such a conserved amino acid is typically found in related TNFs, the Neutrokine-alpha mutein will act as an antagonist, thus possessing activity for example, which inhibits lymphocyte (e.g., B cell) proliferation, differentiation, and/or activation. Accordingly, polypeptides of the present invention include Neutrokine-alpha mutants. Such Neutrokine-alpha mutants comprise, or alternatively consist of, fragments, variants or derivatives of the full-length or preferably the extracellular domain of the Neutrokine-alpha amino acid sequence shown in Figures 1A and 1B (SEQ ID NO:2). Polynucleotides encoding the above Neutrokine-alpha mutants are also encompassed by the invention.

Please replace the paragraph spanning lines 17 to 32 of page 175, with the following amended paragraph:

Since Neutrokine-alphaSV is a member of the TNF-related protein family, to modulate rather than completely eliminate functional activities (e.g., biological activities) of Neutrokine-alphaSV, mutations may be made in sequences encoding amino acids in the TNF conserved domain, i.e., in positions Gly-172 through Leu-265 of Figures 5A and 5B (SEQ ID NO:19), more preferably in residues within this region which are not conserved in all, most or several members of the TNF family (e.g., TNF-alpha, TNF-beta, LT-beta, and Fas Ligand) (see e.g., Figures ~~2A-B~~ **2A, 2B, 2C and 2D**). By making a specific mutation in Neutrokine-alphaSV in the position where such a conserved amino acid is typically found in related TNFs, the Neutrokine-alphaSV mutein will act as an antagonist, thus possessing activity for example, which inhibits lymphocyte (e.g., B cell) proliferation, differentiation, and/or activation. Accordingly, polypeptides of the present invention include Neutrokine-alphaSV mutants. Such Neutrokine-alphaSV mutants comprise, or alternatively consist of, fragments, variants or derivatives of the full-length or preferably the extracellular domain of the Neutrokine-alphaSV amino acid sequence shown in Figures 5A and 5B (SEQ ID NO:19 Polynucleotides encoding the above Neutrokine-alpha SV mutants are also encompassed by the invention.

Please replace the paragraph spanning lines 16 to 33 of page 336, with the following amended paragraph:

A 285 amino acid protein was identified in a human neutrophil/monocyte-derived cDNA library that shared significant homology within its predicted extracellular receptor-ligand binding domain to APRIL (28.7%) (Hahne, M., et al., *J.Exp.Med.* 188,1185-90 (1998)), TNF-alpha (16.2%) (Pennica, D., et al., *Nature* 312,724-729 (1984)) and LT-alpha (14.1%) (Gray, *Nature* 312,721-724 (1984)) (Figure 7A). We have designated this cytokine Neutrokine-alpha (we have also designated this molecule as B Lymphocyte Stimulator (BLyS) based on its biological activity). Hydrophobicity analyses of the the Neutrokine-alpha protein sequence have revealed a potential transmembrane spanning domain between amino acid residues 47 and 73 which is preceded by non-hydrophobic amino acids suggesting that Neutrokine-alpha, like other members of the TNF ligand family, is a type II membrane bound protein (Cosman, D. *Stem.Cells.* 12:440-55 (1994)). Expression of this cDNA in mammalian cells (HEK 293 and Chinese Hamster Ovary) and Sf9 insect cells identified a 152 amino acid soluble form with an N-terminal sequence beginning with the alanine residue at amino acid 134 (arrow in ~~Figure 7A~~ **Figures 7A-1 and 7A-2**). Reconstruction of the mass to charge ratio defined a mass for Neutrokine-alpha of 17,038 Daltons, a value in consistent with that predicted for this 152 amino acid protein with a single disulfide bond (17037.5 Daltons).

Please replace the paragraph spanning lines 6 to 20 of page 337, with the following amended paragraph:

The expression profile of Neutrokine-alpha was assessed by Northern blot (Figure 7B) and flow cytometric analyses (Table V and ~~Figure 8~~ **Figures 8A, 8B and 8C**). Neutrokine-alpha is encoded by a single 2.6kb mRNA found at high levels in peripheral blood leukocytes, spleen, lymph node and bone marrow. Lower expression levels were detected in placenta, heart, lung, fetal liver, thymus and pancreas. Among a panel of cell lines, Neutrokine-alpha mRNA was detected in HL-60 and K562, but not in Raji, HeLa, or MOLT-4 cells. These results were confirmed by flow cytometric analyses using the

Neutrokine-alpha-specific mAb 2E5. As shown in Table V, Neutrokine-alpha expression is not detected on T or B lineage cells but rather restricted to cells within the myeloid origin. Further analyses of normal blood cell types demonstrated significant expression on resting monocytes that was upregulated approximately 4-fold following exposure of cells to IFN-gamma (100 U/mL) for three days (~~Figure 8A~~ **Figures 8A and 8B**). A concomitant increase in Neutrokine-alpha-specific mRNA was also detected (Figure ~~8B~~ **8C**). By contrast, Neutrokine-alpha was not expressed on freshly isolated peripheral blood granulocytes, T cells, B cells, or NK cells.

Please replace the paragraph spanning lines 4 to 21 of page 338, with the following amended paragraph:

In an attempt to correlate the specific biological activity on B cells with receptor expression, purified Neutrokine-alpha was biotinylated. The resultant biotin-Neutrokine-alpha protein retained biological function in the standard B cell proliferation assays. Lineage-specific analyses of whole human peripheral blood cells indicated that binding of biotinylated Neutrokine-alpha was undetectable on T cells, monocytes, NK cells and granulocytes as assessed by CD3, CD14, CD56, and CD66b respectively (~~Figure 10A~~ **Figures 10A, 10B, 10C, 10D and 10E**). In contrast, biotinylated Neutrokine-alpha bound peripheral $CD20^+$ B cells. Receptor expression was also detected on the B cell tumor lines REH, ARH-77, Raji, Namalwa, RPMI 8226, and IM-9 but not any of the myeloid-derived lines tested including THP-1, HL-60, K-562, and U-937. Representative flow cytometric profiles for the myeloma cell line IM-9 and the histiocytic line U-937 are shown in ~~Figure 10B~~ **Figures 10F and 10G**. Similar results were also obtained using a biologically active FLAG-tagged Neutrokine-alpha protein instead of the chemically modified biotin-Neutrokine-alpha. Taken together, these results confirm that Neutrokine-alpha displays a clear B cell tropism in both its receptor distribution and biological activity. It remains to be shown whether cellular activation may induce expression of Neutrokine-alpha receptors on peripheral blood cells, other normal cell types or established cell lines.

Please replace the paragraph beginning on line 30 of page 339 and ending on line 5 of page 340, with the following amended paragraph:

Flow cytometric analyses of the spleens from mice treated with 2 mg/kg Neutrokine-alpha-treated indicated that Neutrokine-alpha increased the proportion of mature ($CD45R(B220)^{dull}$, ThB^{bright}) B cells approximately 10-fold over that observed in control mice (~~**Figure 11B**~~ **Figures 11B and 11C**). Further analyses performed in which mice were treated with buffer, 0.08 mg/kg, 0.8 mg/kg, 2 mg/kg, or 8 mg/kg Neutrokine-alpha indicated that 0.08 mg/kg, 0.8 mg/kg, and 2 mg/kg each increased the proportion of mature ($CD45R(B220)^{dull}$, ThB^{bright}) B cells approximately 10-fold over that observed in control mice, whereas buffer and 8 mg/kg produced approximately equal proportions of mature B cells. *See*, Table IV.

Please replace the paragraph spanning lines 18 to 22 of page 340, with the following amended paragraph:

A potential consequence of increased mature B cell representation *in vivo* is a relative increase in serum Ig titers. Accordingly, serum IgA, IgG and IgM levels were compared between buffer and Neutrokine-alpha-treated mice (~~**Figure 11C**~~ **Figures 11D, 11E, and 11F**). Neutrokine-alpha administration resulted in a 2- and 5-fold increase in IgA and IgM serum levels respectively. Interestingly, circulating levels of IgG did not increase.

In the Claims:

New claims 26 – 359 have been added.